EXHIBIT B

#12
4-4-03

PATENT
Docket No. 524022000100

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3-26-03 Rhea Arnold

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Dexu ZHU, et al

Serial No.: 09/975,136

Filing Date: October 10, 2001

For: METHODS AND COMPOSITIONS FOR TREATING OR PREVENTING BACTERIAL INFECTION

Examiner: Paul A. Zucker

Group Art Unit: 1621

SUPPLEMENTAL DECLARATION OF MING-WEI WANG PURSUANT TO 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Ming-Wei Wang, declare as follows:

1. I am one of the co-inventors of the subject matter claimed in the above-referenced application.

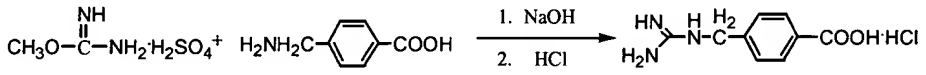
2. I have synthesized (or have directed others to synthesize) the compound I as disclosed in the Satoh et al., U.S. Patent No. 4,732,916 (Satoh) and its related intermediate

compounds. I have compared (or have directed others to compare) anti-*H. pylori* activity of one of the claimed compounds of the present application (NE-2001, see claim 2 of the present application) and the compound I as disclosed in Satoh and its related intermediate compounds. The comparison indicates that, unlike NE-2001, the compound I as disclosed in Satoh and its related intermediate compounds hardly have any anti-*H. pylori* activity. The chemical synthesis and anti-*H. pylori* activity study are set forth in detail in the following paragraphs 3-15.

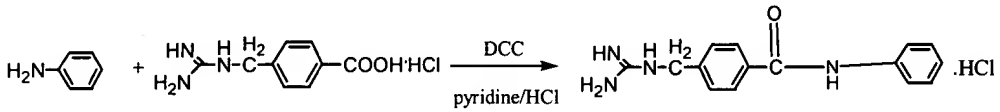
Synthesis of N-Phenyl-(4-guanidinomethyl) benzoyl amide hydrochloride (Satoh Compound)

3. Scheme of synthesis

1



2



4. Instruments and reagents

HP1100 HPLC system includes binary pump, on-line degasser, auto-sampler, thermostatted column compartment and diode-array detector. The column is ZORBAX ODS (4.6 x 250 mm). Mobile phase is methanol/water = 80:20 (0.1% acetic acid). Flow rate is 1 ml/min. The detector wave is 254 nm. All solvents are HPLC grade. MS spectra are obtained by API 2000 LC/MS/MS system. All starting materials are available in the market.

5. 4-Guanidinomethyl benzoic acid hydrochloride

Two (2) N NaOH solution (72 ml) was added to a solution of methyl isothiourea disulfate 20.0 g (0.140 mol) in 36 ml water with cooling in ice batch, and stirred. Then 21.0 g (0.138 mol) 4-aminomethylbenzoic acid in 140 ml 2 N NaOH solution was added dropwise. The mixture was stirred overnight at room temperature and then chilled in ice water for 1 hour. The precipitated white crystals were filtered off and washed with cold water. The filtrate was dissolve in warm 1 N HCl and insoluble material was removed by filtration. The solution was concentrated in vacuum to crystallize. The colorless prisms crystallized when the solution was cooled, then filtered and dried, gave 4-guanidinomethyl benzoic acid hydrochloride 22.1 g (yield 70%). LC/MS = 194 (M+H)

6. N-Phenyl-(4-guanidinomethyl) benzoyl amide hydrochloride

A suspension of Aniline 1.0 g (0.010 mol), 4-Guanidinomethyl benzoic acid hydrochloride 2.3 g (0.010 mol) and dicyclohexylcarbodiimide 4.1 g (0.020 mol) in pyridine (150 ml) was stirred at room temperature for 48 hours, after removal of insoluble materials by filtration. The filtrate was evaporated to dryness and residue solid was treated with 1 N hydrochloric acid (50 ml) and ether (50ml), the aqueous layer was washed with ether again and concentrated to 20 ml, the resulting crystals were recrystallized in water, gave N-Phenyl-(4-guanidinomethyl) benzoyl amide hydrochloride 1.9 g (yield 70%). LC/MS = 269 (M+H)

Method of detection of minimum inhibitory concentration (MIC) of anti-*H. pylori* activity

7. Sensitivity measuring method

Sensitivity was measured by an agar plating dilution method using Columbia agar (Difco Co.) that was added with 5% defibrinated sheep blood.

8. Concentration stepwise of anti-bacterial agent NE-2001

The prepared concentrations stepwise were 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06 and 0.03 μg/ml. A solution of the NE-2001 with a concentration of 6.4 mg/ml was obtained from the present invention and was prepared by dissolving in 1% (2-Hydroxypropyl)-b-cyclodextrin solution (Sigma). After sterilization, the solution was diluted with sterilized water to 640, 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25, 0.6 and 0.3 μg/ml. Each of 1 ml of those prepared solutions was dispensed in 9 ml of sensitivity measuring medium that was kept at 50°C and was added to each, and they were sufficiently mixed. The mixture was used as a plate for measuring sensitivity.

9. Bacteria solution for inoculation

Brucella broth (BB), which was added with 10% fetal bovine serum, was used as bacteria growing medium, and was cultured under microaerophilic condition (5% O_2, 10% CO_2 and 85% N_2) at 37°C for 24 hours, and the number of bacteria thereof was adjusted to about 10^8 CFU/ml. This was used as a bacterial solution for inoculation.

10. Method of inoculating bacteria

The bacteria solution was painted in each square about 2 cm with inoculating line loop.

11. Period and temperature of the culture

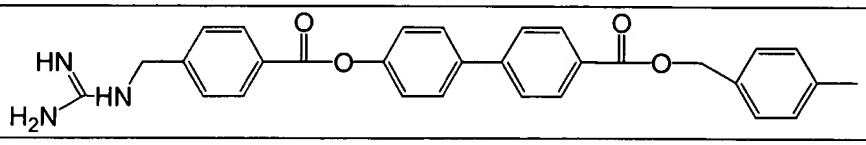
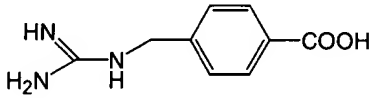
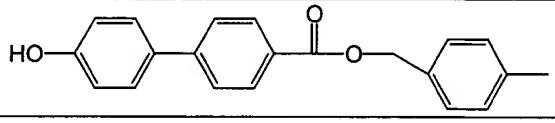
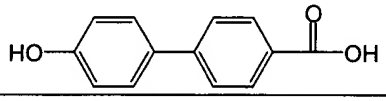
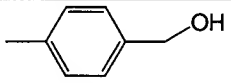
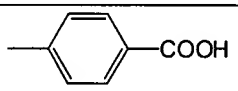
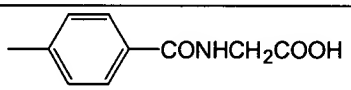
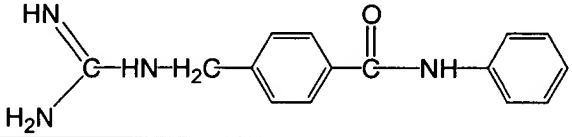
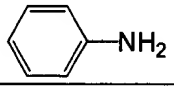
The plates were cultured under microaerophilic condition for three days at 37°C.

12. Evaluation

The minimum concentration that completely inhibits the growth of the medium was judged as the value of MIC.

Anti-*H. pylori* activity

13. Anti-*H. pylori* activities of NE-2001 and the compound I as disclosed in Satoh and its related intermediate compounds were assayed as described above and the assay results are set forth in the following Table 1.

Compound	Structure	MIC Value	Anti-*H. pylori* activity
NE-2001		2 μg	100%
Intermediate A		32 μg	6.25%
Intermediate B		28 μg	7.14%
Intermediate C		32 μg	6.25%
Intermediate D		>64 μg	0
Related Compound A		>64 μg	0
Related Compound B		>64 μg	0
Satoh Compound		64 μg	3.13%
Aniline		>64 μg	0

14. NE-2001 is a four-benzene ring compound linked by two ester bonds; the compound described in the Satoh patent is a two-benzene ring compound linked by an amide-bond. The linkage between these two compounds is entirely different. Therefore, their molecular structures and associated physicochemical prosperities are distinctly different.

15. The above structure-activity study indicates that the Intermediates A, B, C linked by ester-bond have some anti-*H. pylori* activities compared to NE-2001; while Satoh Compound and Related Compound B, both linked by amide-bond, have little or no anti-*H. pylori* efficacy. These results suggest that the anti-ulcer activity allegedly disclosed in Satoh's patent has no relationship with the anti-*H. pylori* activity of NE-2001. Our experimental data further indicate that NE-2001 linked by ester-bonds with benzene rings significantly enhances the anti-*H. pylori* activity, while the same intermediate linked by an amide-bond with benzene-rings has very little anti-*H. pylori* property, thus distinguishing NE-2001 from Satoh Compound not only in their molecular structures, but also in their anti-*H. pylori* bioactivities.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at San Diego, California, on February 10, 2003.

Ming-Wei Wang